# Capsular Complexes of Nonpolar Guests with Octa Amine Host Detected in the Gas Phase

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**ABSTRACT**
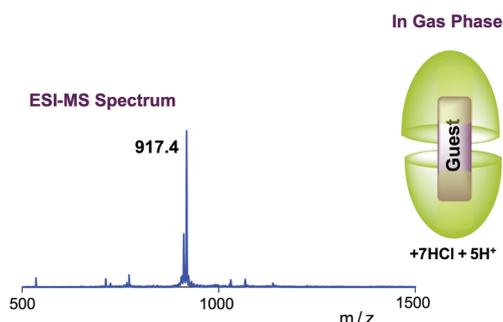


Nanocapsules, made up of the deep cavitand octa amine and several guests, were prepared in aqueous acidic solution and were found to be stable in the gas phase as detected by electrospray ionization mass spectrometry (ESI-MS). The observed gas phase host−guest complexes contained five positive charges and were associated with several acid molecules (HCl or HBr).

During the past three decades a number of cavitands have been explored in the context of host−guest complexation in solution. Occasionally these cavitands, prompted by guest molecules, self-assemble to form a closed container (capsule).[1] These capsular assemblies termed capsuleplexes are stabilized in solution through noncovalent interactions such as hydrogen bonding, metal coordination, electrostatic interactions, and hydrophobic effects.[2]

Despite the recognized potential of water-soluble hosts for biorelated applications,[3] there are relatively few supramolecular cavitands that assemble to form capsular assemblies in water.[4] One of the early examples of a water-soluble closed capsule was provided by Cram.[5] Due to its closed nature and small size, its use as a reaction cavity was limited. More useful water-soluble capsules that are generated through self-assembly of cavitands became available through the efforts of Rebek,[6] Reinhoudt,[7] and Gibb.[8]

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(1) (a) Schmuck, C. *Angew. Chem., Int. Ed.* **2007**, *46*, 5830. (b) Lutzen, A. *Angew. Chem., Int. Ed.* **2005**, *44*, 1000.
(2) Ninham, B. W.; Lo Nostro, P. *Molecular Forces and Self-Assembly*; Cambridge University Press: Cambridge, 2010.
(3) Oshovsky, G. V.; Reinhoudt, D. N.; Verboom, W. *Angew. Chem., Int. Ed.* **2007**, *46*, 2366.

(4) (a) Brinker, U. H.; Mieusset, J.-L. *Molecular Encapsulation*; John Wiley & Sons, Ltd.: Chichester, 2010. (b) Conn, M. M.; Rebek, J. *Chem. Rev.* **1997**, *97*, 1647.
(5) Yoon, J.; Cram, D. J. *Chem. Commun.* **1997**, 497.
(6) Hooley, R. J.; Van Anda, H. J.; Rebek, J. *J. Am. Chem. Soc.* **2007**, *129*, 13464.
(7) Corbellini, F.; Knegtel, R. M. A.; Grootenhuis, P. D. J.; Crego-Calama, M.; Reinhoudt, D. N. *Chem.—Eur. J.* **2005**, *11*, 298.
(8) Gibb, C. L. D.; Gibb, B. C. *J. Am. Chem. Soc.* **2004**, *126*, 11408.

Recently we reported a cavitand with internal features similar to those of Gibb's octa acid[8] but with different external pendant groups.[9] Ours had amine groups at the periphery that made the cavitand soluble in water under acidic conditions. We have established during the past few years the exceptional value of Gibb's octa acid and our octa amine (OAm; Scheme 1) as photochemical reaction vessels in water.[9,10] Our recent interest in exploring the stability of the capsular assemblies in the gas phase has led us to carry out a detailed electrospray mass spectrometry study (ESI-MS) of host−guest systems involving OAm. The results reported here provide new physical insight into the capsular formation and stability in the gas phase.

**Scheme 1.** Structure of OAm (Host) and of the Guests **1**−**8**
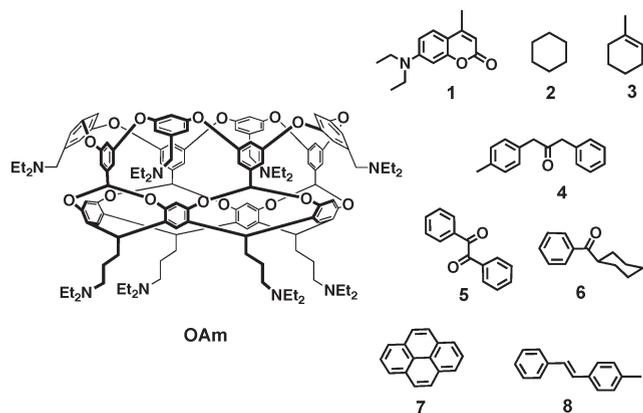


The ESI-MS technique has been recognized as a powerful tool to study the stoichiometry and interactions of macromolecular assemblies in general[11,12] and host−guest complexes (caviplexes) and capsular assemblies (capsuleplexes) in particular.[13] In spite of this, to our knowledge, there are no examples of stable capsuleplexes in the gas phase that are stabilized primarily by hydrophobic forces in solution. ESI-MS is useful when the self-assembly is driven by electrostatic or hydrogen bond interactions.[13,14] Since the solvent and the entropy-driven hydrophobic effect are absent in the gas phase[13,15] such capsuleplexes are expected

to be unstable in the gas phase. This study presents several examples of detectable capsuleplexes in the gas phase that are stabilized in solution predominantly by hydrophobic forces.

The cavitand that we reported recently known as octa amine (OAm) is soluble in water under acidic conditions (HCl or HBr) and forms 1:1, 2:1, or 2:2 complexes with organic guest molecules.[9] In this study we are interested in capsuleplexes in which one or two guest molecules are enclosed within a capsule made up of two molecules of OAm. The host OAm forms capsular assemblies with the guests **1**−**8** shown in Scheme 1. By 1-D and 2-D ^{1}H NMR studies we had shown earlier that the guests **1**, **4**, and **7** form 2:1 capsuleplexes.[9] With the help of the photophysical probe **7** we inferred that the interior of the capsule is nonpolar. In the current study we report that the guests **2** and **3** form 2:2 and **5**, **6** and **8** form 2:1 capsuleplexes. Typical ^{1}H NMR spectra of 2:2 and 2:1 capsular assemblies are shown in Figure 1 with guests **2** and **6** as prototypical examples. ^{1}H NMR spectra of the other guests are shown in Figures S1−S8 in the Supporting Information (SI).

An upfield shift of the guest proton signals prompted by diamagnetic shielding by the aromatic groups that form the cavity interior generally suggests inclusion of guest molecules within the capsule of OAm. ^{1}H NMR titration experiments suggested the formation of capsular assemblies in the presence of the guests listed in Scheme 1 in aqueous acidic solution. Our curiosity to examine the stability of such complexes in the gas phase prompted the current investigation. For this purpose we have employed ESI-MS as a tool. We begin the discussion with the detection of OAm in the gas phase and follow this with the results on capsuleplexes.
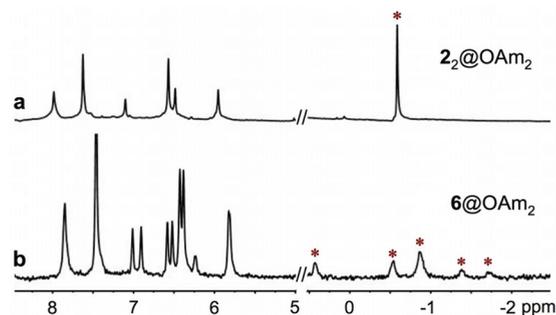


**Figure 1.** ^{1}H NMR spectra (500 MHz) of (a) $\mathbf{2}_2$@OAm$_2$ and (b) $\mathbf{6}$@OAm$_2$ ([OAm] = 1 mM, [**2**] = 1 mM, [**6**] = 0.5 mM) in D$_2$O/DCl (pH ≈ 1). Bound guest signals are marked with red *.

Under soft ionization conditions (capillary exit potential 100 V, skimmer potential 5 V, desolvation temperature 100 °C; see SI for experimental details) the ESI-MS of a 100 $\mu$M acidic aqueous solution (HCl, 0.37%) of OAm provided signals at $m/z$ 533.8, 711.1, and 1066.2, with four ($\Delta m = 0.25$), three ($\Delta m = 0.33$), and two positive charges ($\Delta m = 0.5$), respectively (Figure 2).

(9) Kulasekharan, R.; Ramamurthy, V. *Org. Lett.* **2011**, *13*, 5092.

(10) (a) Sundaresan, A. K.; Gibb, C. L. D.; Gibb, B. C.; Ramamurthy, V. *Tetrahedron* **2009**, *65*, 7277. (b) Gupta, S.; Choudhury, R.; Krois, D.; Wagner, G.; Brinker, U. H.; Ramamurthy, V. *Org. Lett.* **2011**, *13*, 6074. (c) Kulasekharan, R.; Choudhury, R.; Prabhakar, R.; Ramamurthy, V. *Chem. Commun.* **2011**, *47*, 2841.

(11) Hernández, H.; Robinson, C. V. *Nat. Protoc.* **2007**, *2*, 715.

(12) Kogej, K.; Schalley, C. A. In *Analytical Methods in Supramolecular Chemistry*; Schalley, C. A., Ed.; John Wiley & Sons: Chichester, 2007.

(13) (a) Pemberton, B. C.; Singh, R. K.; Johnson, A. C.; Jockusch, S.; Da Silva, J. P.; Ugrinov, A.; Turro, N. J.; Srivatsava, D. K.; Sivaguru, J. *Chem. Commun.* **2011**, *47*, 6323. (b) Da Silva, J. P.; Jayaraj, N.; Jockusch, S.; Turro, N. J.; Ramamurthy, V. *Org. Lett.* **2011**, *13*, 2410. (c) Weimann, D. P.; Schalley, C. A. *Supramol. Chem.* **2008**, *20*, 117. (d) Rozhenko, A. B.; Schoeller, W. W.; Letzel, M. C.; Decker, B.; Agena, C.; Mattay, J. *Chem.—Eur. J.* **2006**, *12*, 8995.

(14) (a) Zadmard, R.; Kraft, A.; Schrader, T.; Linne, U. *Chem.—Eur. J.* **2004**, *10*, 4233. (b) Oshovsky, G. V.; Reinhoudt, D. N.; Verboom, W. *J. Org. Chem.* **2006**, *71*, 7441.

(15) Bich, C.; Baer, S.; Jecklin, M. C.; Zenobi, R. *J. Am. Soc. Mass Spectrom.* **2010**, *21*, 286.

Under harder ionization conditions (capillary exit potential 320 V, skimmer potential 100 V, desolvation temperature 300 °C; see SI for experimental details) the mass spectrum of OAm changed to an envelope of ions at $m/z$ 2059.2, 1030.0, 687.0, 515.6, and 412.9, with one, two, three, four, and five positive charges, respectively (Figure S9 in SI). The charge state of each ion was confirmed by Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS). Based on the molecular mass of OAm and on the observed charge states, the ions at $m/z$ 2059.2, 1030.0, 687.0, 515.6, and 412.9 (Figure S9 in SI) were readily assigned to $[OAm+H]^+$, $[OAm+2H]^{2+}$, $[OAm+3H]^{3+}$, $[OAm+4H]^{4+}$, and $[OAm+5H]^{5+}$, respectively.

A comparison of the spectra obtained under soft and hard ionization conditions (Figure 2 and Figure S9 in SI) shows that the doubly charged ion observed under the former conditions ($m/z$ 1066.2) had an $m/z$ increase of 36 units (mass 72 and charge +2) relative to the one observed under harder conditions ($m/z$ 1030.0). The other two ions, the triply and quadruply charged ($m/z$ 711.1 and 533.8), also showed an additional 72 Da.
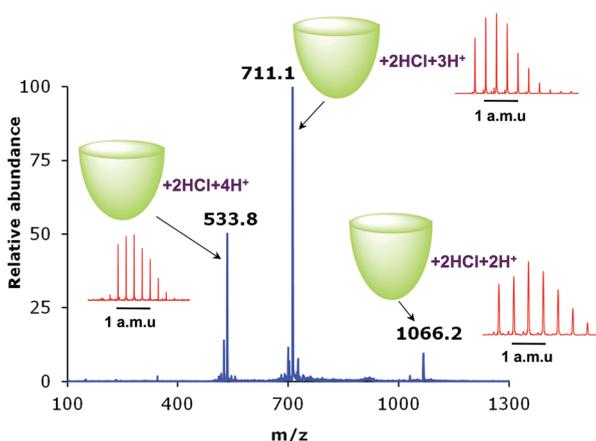


**Figure 2.** ESI-MS spectrum (full scan) of an aqueous solution of OAm (100 $\mu$M) with HCl (0.37%). Desolvation temperature 100 °C, capillary exit 100 V, skimmer 5 V. The insets show the isotope patterns at each $m/z$ value determined by FT-ICR-MS. Assignments: $m/z$ 533.8 $[OAm+2HCl+4H]^{4+}$; $m/z$ 711.1 $[OAm+2HCl+3H]^{3+}$; $m/z$ 1066.2 $[OAm+2HCl+2H]^{2+}$.

The fragmentations (MS-MS) of $m/z$ 1066.2 lead to $m/z$ 1048.2 and 1030.2, indicating neutral losses of 36 and 72 Da, respectively (Figure S10a in SI). The same losses were observed after fragmentation of the ions observed at $m/z$ 711.1 and 533.8 (Figure S10b and c). All fragmentations resulted in the same ion observed under hard ionization conditions (Figure S9 in SI). The observed mass change could be accounted for by the loss of two HCl molecules, four $H_2O$ molecules, or a combination of both chemical species. The definitive assignments of the ions observed under soft ionization conditions (Figure 2) were made following the use of a solution of OAm prepared with $D_2O$ and DCl (Figure S11 in SI). The masses of the main

ions from this solution, within experimental error, shifted by 6 Da ($m/z$ 533.8), 5 Da ($m/z$ 711.1), and 4 Da ($m/z$ 533.8), which can be accounted for by the presence of $2DCl+4D^+$, $2DCl+3D^+$, and $2DCl+2D^+$, respectively (compare Figure 2 and Figure S11 in SI). This indicated that the OAm cavitand came into the gas phase with two HCl molecules. We therefore assign the ions at $m/z$ 533.8, 771.1, and 1066.2 to $[OAm+2HCl+4H]^{4+}$, $[OAm+2HCl+3H]^{3+}$, and $[OAm+2HCl+2H]^{2+}$, respectively. These assignments are in agreement with the isotope patterns obtained by FT-ICR-MS (Figure S12 in SI). Experiments conducted with aqueous solutions of OAm with HBr (0.47%) also formed OAm ions containing HBr molecules (Figure S13 in SI).

Having established the ESI-MS behavior of the host OAm, we proceeded to perform the ESI-MS studies of capsuleplexes of OAm with guests **1−8** whose presence in aqueous acidic solution, as mentioned above, was established by $^1H$ NMR. As an example, a detailed analysis of mass spectra of **1**@OAm$_2$ is presented below. The mass spectra of the complexes of **2−8** with OAm are presented in the SI, and interpretation of these follows the same reasoning used for **1**@OAm$_2$, which allowed us to conclude that capsuleplexes of OAm with guests **1−8** are stable in the gas phase.

Complexes of **1**@OAm$_2$ gave a mass spectrum with a base peak at $m/z$ 921.3 and a minor signal at 232.0 (Figure 3a). The weak signal at 232.0 was assigned to the free protonated guest. The isotope pattern near $m/z$ 921 (Figure 3b) revealed ions with five positive charges ($\Delta m$ = 0.2). The fragmentation of $m/z$ 921.3 resulted mainly in free OAm, with three or four charges and containing one or two HCl molecules (Figure S14 in SI). The free guest is also observed after fragmentation ($m/z$ 232.0), indicating that the aggregate detected at $m/z$ 921.3 contained both the host and the guest. Based on the $m/z$ value, on the charge state, on the fragmentation patterns and on the behavior of the free OAm (see Figure 2 and Figure S10 in SI), the ion observed at $m/z$ 921.3 should correspond to a capsule containing two OAm and one molecule of **1** (2:1 host−guest complex) and *seven* HCl molecules.

The results for **1**@OAm$_2$ prepared in $D_2O$ and with DCl gave an $m/z$ value for the aggregate shifted by 2.5 relative to the samples prepared in $H_2O$ and with HCl (Figure S15 in SI). Since the charge state of the ion is five, the shift corresponded to ∼12 Da. Seven DCl molecules plus 5 $D^+$ ions account for the observed shift. We therefore assign the ion at $m/z$ 921.3 to $[\mathbf{1}@OAm_2+7HCl+5H]^{5+}$. The experimental and the simulated isotope patterns, together with high accuracy mass measurements, both determined by FT-ICR-MS, were in agreement with the assigned structure of the capsule. FT-ICR-MS generates near $m/z$ 921 an isotope distribution shown in Figure 3b. Spectra provided in Figure 3c and 3d show the simulated isotope profiles of the ions $[\mathbf{1}@OAm_2+7HCl+5H]^{5+}$ and $[\mathbf{1}@OAm_2+14H_2O+5H]^{5+}$. Consistent with the above conclusion, the simulated spectrum for $[\mathbf{1}@OAm_2+7HCl+5H]^{5+}$ fits the experimental one. Furthermore, the exact mass for the first peak of the isotope series is 920.45289, which is

consistent with the formula $C_{270}H_{333}O_{34}N_{17}Cl_7$, the molecular formula of the ion $[1@OAm_2+7HCl+5H]^{5+}$.
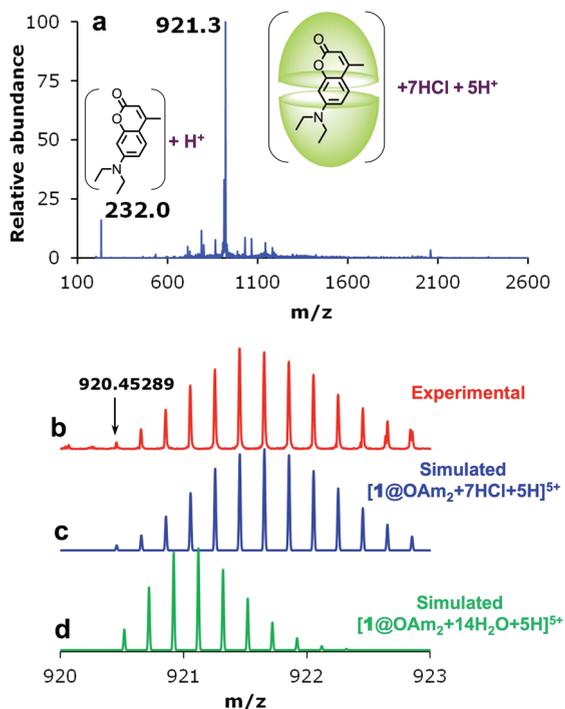


**Figure 3.** ESI-MS spectra of $1@OAm_2$. (a) Full scan spectrum; (b) experimental FT-ICR-MS spectrum near $m/z$ 921; (c) simulated isotope pattern for $[1@OAm_2+7HCl+5H]^{5+}$; and (d) simulated isotope pattern for $[1@OAm_2+14H_2O+5H]^{5+}$. Desolvation temperature 100 °C, capillary exit 100 V, skimmer 5 V. Assignments: $m/z$ 232.0 $[1+H]^+$; $m/z$ 921.3 $[1@OAm_2+7HCl+5H]^{5+}$. The exact mass (920.45289) generates the molecular formula $C_{270}H_{333}O_{34}N_{17}Cl_7$.

The mass spectra of the complexes of **2−8** with OAm recorded under soft conditions are provided in Figures S16 ($2_2@OAm_2$), S17 ($3_2@OAm_2$), S18 ($4@OAm_2$), S19 ($7@OAm_2$), S20 ($5@OAm_2$), S21 ($6@OAm_2$), and S22 ($8@OAm_2$) in the SI. In all cases the base peak is the ion

corresponding to the capsuleplex with 7 HCl and 5 H$^+$. At this stage we are unable to offer any explanation for the preference of 7 HCl and 5 H$^+$. The capsule has 16 NEt$_2$ groups, and of these, apparently, only 12 are protonated in the gas phase.

It is important to point out that in the gas phase we did not detect empty capsules, *i.e.*, two associated host molecules without any guest within. Even in solution, the presence of empty capsules was not evident in the $^1$H NMR spectrum. These observations confirm that capsule formation in solution is guest induced and driven by hydrophobic effects. The detection of stable 2:1 and 2:2 guest@OAm complexes without water molecules surrounding them in the gas phase suggests that interactions other than the hydrophobic effect should contribute to capsule stability in the gas phase.[15] Since the capsules are detected in the gas phase with seven HCl molecules, the ion−ion interactions involving the protonated amine groups of the hosts and the external chloride ions are likely to be an important attractive force that keeps the capsules stable in the gas phase.[16,17] In summary, we have demonstrated (a) the use of ESI-MS to prepare and detect stable OAm capsules in the gas phase, (b) gas phase OAm complexes are seen as five charged ions with seven acid (HCl/HBr) molecules, and (c) the involvement of ion−ion interaction in capsule stability in the gas phase.

**Supporting Information Available.** Sample preparation procedures and additional NMR and MS data. This material is available free of charge via the Internet at http://pubs.acs.org.

(16) Rehm, T. H.; Schmuck, C. *Chem. Soc. Rev.* **2010**, *39*, 3597.

(17) Arunachalam, M.; Ghosh, P. *Chem. Commun.* **2009**, 3184.